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Research Article

EVALUATION OF ACUTE ORAL TOXICITY OF A POLYHERBAL AYURVEDIC FORMULATION IN WISTAR RATS AS PER OECD 423

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ABSTRACT

The present study systematically evaluated the acute oral toxicity profile of Amrith Noni Artho Plus - a polyherbal Ayurvedic formulation containing *Morinda citrifolia* (Noni), *Vitex negundo (Nirgundi), Boswellia serrata (Shallaki),* and *Commiphora wightii* (*Guggulu*) - in accordance with OECD Guideline 423. Twelve female Wistar rats were subjected to a stepwise testing protocol involving initial (Step 1) and confirmatory (Step 2) phases, with three animals per dose group receiving single oral administrations of either 300mg/kg or 2000mg/kg body weight (limit test dose) via gavage. The formulation was suspended in normal saline (10ml/kg) and administered following overnight fasting. Animals underwent intensive clinical monitoring at predefined intervals (10 min, 30 min, 1h, 2h, 4h, 6h) post-dosing followed by twice-daily observations for 14 days. No mortality or treatment-related clinical signs of toxicity (including behavioural alterations, neurological symptoms, or autonomic disturbances) were observed at any dose level. Body weight trajectories and food/water consumption patterns remained normal throughout the study period, with no statistically significant deviations from baseline ($p > 0.05$). Gross pathological examination upon termination revealed no abnormalities in vital organs (liver, kidneys, heart, and spleen), and organ-to-body weight ratios fell within normal physiological ranges. The absence of adverse effects at 2000mg/kg classifies this formulation as Category 5 ("unclassified") under the OECD Globally Harmonized System, indicating an exceptionally wide safety margin. This study demonstrates that Amrith Noni Artho Plus is safe at doses up to 2000mg/kg, supporting its non-toxic nature and potential for safe therapeutic use.

INTRODUCTION

The use of polyherbal Ayurvedic formulations has gained significant attention in recent years due to their potential therapeutic benefits and relatively fewer side effects compared to synthetic drugs.[1] Ayurveda, the traditional Indian system of medicine, relies on natural plant-based remedies to treat various ailments, often combining multiple herbs to enhance efficacy and bioavailability through their synergistic interactions.[2] Among these, *Morinda citrifolia* (Noni) has been extensively studied for its anti-inflammatory, antioxidant, and immunomodulatory properties.[3,4]

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When combined with other well-known Ayurvedic herbs such as *Vitex negundo* (*Nirgundi*), *Boswellia serrata* (*Shallaki*), and *Commiphora wightii* (*Guggulu*), the resulting formulation may offer enhanced therapeutic effects, particularly in managing inflammatory disorders.

Despite the widespread use of such herbal formulations, scientific validation of their safety remains crucial, especially concerning acute toxicity profiles. Regulatory agencies, including the World Health Organization (WHO) and the Organization for Economic Co-operation and Development (OECD), emphasize the need for standardized toxicity testing to ensure consumer safety.[5] The OECD Guideline 423 (Acute Oral Toxicity– Acute Toxic Class Method) provides a well-established protocol for assessing the potential hazards of test substances, classifying them based on lethality and observed adverse effects. This guideline is particularly useful for evaluating herbal

medicines, as it minimizes animal usage while providing reliable toxicity data.

Amrith Noni Artho Plus is a polyherbal Ayurvedic formulation primarily composed of *M. citrifolia* along with other key ingredients such as *Nirgundi, Shallaki, Guggulu* and so on. These herbs have been traditionally used for their anti-arthritic, anti-inflammatory, and analgesic properties.[6-8] However, despite their therapeutic potential, comprehensive toxicity studies are essential to establish their safety profile, particularly concerning acute exposure. The safety of their combined formulation remains to be systematically evaluated. Herbal synergism, while often enhancing therapeutic efficacy, may also alter pharmacokinetics and toxicity, necessitating rigorous assessment.[9] Polyherbal formulations introduce complexities such as herb-herb interactions, which could potentially lead to unexpected adverse effects or altered toxicity thresholds.[10]

Acute oral toxicity testing is the first step in assessing the safety of any new formulation, providing critical data on the lethal dose (LD_{50}), clinical signs of toxicity, and possible target organ damage.[11] Considering the increasing consumer dependence on herbal medicines, it is important to ensure that such formulations do not pose significant health risks even at higher doses. The present study aimed to evaluate the acute oral toxicity of Amrith Noni Artho Plus in Wistar rats following OECD Guideline 423.

MATERIALS AND METHODS

Chemical and reagents

All chemicals and reagents used in the acute oral toxicity study were of analytical grade and procured from Sigma-Aldrich (St. Louis, Missouri, USA). The standard Nutrimix laboratory rat pellet diet (supplied by Nitrivet Life Science, Pune, Maharashtra) was provided ad libitum to maintain uniform nutritional conditions throughout the study. The test formulation, Amrith Noni Artho Plus, was supplied by VALYOU Products Pvt. Ltd., Karnataka, India.

Collection of plant materials

The dry materials used in the formulation, including *M. citrifolia*, *V. negundo*, *B. serrata*, *C. wightii*, and others were procured from an authenticated local vendor specializing in medicinal herbs. The plant materials were carefully selected based on their morphological characteristics and were verified for authenticity by a qualified botanist. The collected materials were stored in airtight containers under controlled temperature and humidity conditions until further processing.

Preparation of polyherbal formulation

The polyherbal Ayurvedic formulation Amrith Noni Artho Plus was prepared as an aqueous suspension for oral administration. The dried plant materials were finely powdered and mixed in their traditional proportions. For the study, a calculated quantity of the herbal blend was suspended in sterile normal saline (0.9% NaCl) to achieve the desired test concentrations (e.g., 2000mg/kg for limit test). The suspension was freshly prepared before each administration and homogenized using a magnetic stirrer to ensure uniform distribution of herbal particles. The mixture was then sonicated for 15 minutes to enhance solubility and administered to animals within 1 hour of preparation to maintain consistency. Each 15 ml of Amrith Noni Artho Plus contained the following ingredients: *M. citrifolia* (juice, 8ml), *V. negundo* (juice, 1ml), *B. serrata* (100mg), *C. wightii* (250mg), *Pluchea lanceolata* (*Rasna*, 450mg), *Tribulus terrestris* (*Gokshura*, 250mg), *Aegle marmelos* (*Bael*, 80 mg), *Ricinus communis* (*Eranda*, 450mg), *Sida cordifolia* (*Bala*, 100mg), *Dashamoola* (250mg), *Barleria prionitis* (*Sahachara*, 150mg), *Garcinia cambogia* (*Vrikshamla*, 100mg), *Rubia cordifolia* (*Manjistha*, 250mg), *Plumbago zeylanica* (*Chitrak*, 100mg), and small quantities of other herbs, including *Premna serratifolia (Agnimantha), Gmelina arborea (Gambhari), Oroxylum indicum (Shyonaka), Stereospermum suaveolens (Patala), Solanum indicum (Brihati), Desmodium gangeticum (Shalaparni), Solanum xanthocarpum (Kantakari),* and *Uraria picta (Prishniparni).*

Acute Oral Toxicity Study

Experimental animals and ethics committee

The acute oral toxicity study was conducted using healthy, nulliparous, non-pregnant female Wistar rats (n=12), bred in-house under controlled environmental conditions. The animals, aged 8-12 weeks with a body weight 180-240 grams, were acclimatized for 6 days prior to the experiment under standard laboratory conditions. The study protocol (Approval No: ABS-IAEC-102-2023-24) was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Apollo Biosciences in compliance with OECD Guideline 423 and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. During the study, animals had *ad libitum* access to Nutrimix laboratory pellet diet and filtered water.

Animal Acclimatization and husbandry conditions

The animals were housed in standard polypropylene cages (three rats per cage) with stainless steel mesh tops, containing separate provisions for pellet feed (Nutrimix laboratory diet) and drinking water. The animal facility maintained

strict environmental controls, including air-conditioning with 12-15 fresh air changes per hour, ambient temperature of 18-29°C, relative humidity of 30-70%, and a 12-hour light/dark cycle.

Before to the study, the rats were acclimatized with the laboratory conditions for 6-days. Body weights were recorded on the first day of acclimatization and again before dosing. Daily health monitoring was conducted to assess morbidity and mortality, and only healthy, active animals were selected for the study. The rats were fasted overnight (12-16 hours) before dosing, with food reintroduced 3 hours post-administration.

Animal groups and doses

A total of 12 female Wistar rats were randomly divided into four experimental groups (n=3 per group) for dose administration following OECD Guideline 423. All animals received Amrith Noni Artho Plus via oral gavage (p.o.) at standardized volumes of 10ml/kg body weight. Group 1 and Group 2 were orally administered 300mg/kg of the test formulation, while Group 3 and Group 4 received the higher dose of 2000mg/kg (limit test dose). The experiment was repeated twice (two independent replicates). Animals were fasted overnight prior to dosing, and the suspension was freshly prepared in normal saline (0.9% NaCl) and homogenized before each administration to ensure dose consistency.

Experimental procedure

The acute oral toxicity study was conducted as per OECD Guideline 423 using a stepwise design with three female Wistar rats per step. The test formulation, Amrith Noni Artho Plus, was administered as a single oral dose via gavage using a graduated syringe fitted with an oral intubation needle. Prior to dosing, animals were fasted for 12-16 hours (with free access to water), and individual doses were adjusted to maintain a constant administration volume of 10ml/kg body weight based on pre-dose weight measurements. Following administration, animals were closely monitored for signs of toxicity, morbidity, and mortality at specified intervals (10 min, 30 min, 1h, 2h, 4h, and 6h of post-dosing), followed by daily observations for 14 days. Food was reintroduced 3 hours post-dosing to standardize metabolic conditions. The study design included duplicate dose groups (300 and 2000mg/kg) to ensure robust toxicity assessment, with all procedures conducted under controlled laboratory conditions to minimize confounding variables.

Observations

Food consumption

Daily food consumption was measured throughout the 14-day study period. Consumption patterns were documented to assess appetite changes or aversion behaviours. Measurements were standardized to account for diurnal variations and recorded at consistent times each day.

Body weight

Body weights were recorded pre-dosing (day 0) and post-dosing on days 1, 8, and 15. Weight measurements were conducted using calibrated digital scales, and percentage changes from baseline were calculated to evaluate potential effects on growth or metabolic function.

Clinical signs and mortality

Following administration of Amrith Noni Artho Plus, all animals were closely monitored for clinical signs of toxicity at predetermined intervals (10 min, 30 min, 1h, 2h, 4h, and 6h of post-dosing) and subsequently twice daily for 14 days. Observations focused on behavioural changes (activity levels, grooming, posture), neurological symptoms (tremors, convulsions), autonomic responses (salivation, lacrimation), and physical condition (fur quality, eye clarity). Mortality checks were performed during each observation interval to assess acute lethality.

Necropsy and gross pathology

On day 14, all animals were humanely euthanized through CO_2 asphyxiation followed by cervical dislocation, after which a thorough gross pathological examination was conducted. All major organs, including the liver, kidneys, heart, and spleen were carefully examined for any macroscopic abnormalities. The evaluation specifically focused on detecting discoloration, morphological changes (such as swelling or atrophy), visible lesions (including nodules or haemorrhages), and any size or weight discrepancies.

Statistical analysis

All experimental data were analyzed using GraphPad Prism software (version 5.0, Boston, MA). Quantitative parameters including body weights were expressed as mean ± standard deviation (SD), while clinical signs and symptoms were analyzed using both descriptive statistics. The incidence and frequency of clinical observations (e.g., lethargy, tremors, autonomic effects) were documented. For longitudinal comparisons, post-treatment time points (days 1, 3, 7, and 14) were compared against day 0 (pre-dose) values using repeated measures ANOVA with Dunnett's multiple comparison test. A p-value <0.05 was considered statistically significant.

RESULTS

Food consumption

No significant alterations in food or water intake were observed in any treatment group throughout the 14-day observation period. Animals administered Amrith Noni Artho Plus at both 300 and

2000 mg/kg doses maintained consistent consumption patterns comparable to baseline measurements.

Body weight

All animals demonstrated normal progressive weight gain at both dose levels during the 14-day study period, with statistically significant increases compared to baseline (day 0) measurements. In the 300mg/kg group, mean body weight increased significantly from 188.77±2.27g (day 0) to 200.83± 6.73g by day 8 ($p < 0.01$) and 220.67±5.32 g by day 14 ($p < 0.001$). Similarly, the 2000mg/kg group showed weight gain from 195.54±11.44 g (day 0) to 207.50± 10.80g (day 8) and 223.42±10.24 g (day 14, $p < 0.001$). Percentage weight gain calculations revealed comparable growth patterns between groups, with increases of 6.37±2.45% (300mg/kg) and 6.18±2.65% (2000mg/kg) by day 8, and increased to 16.89±2.41% and 14.36±3.38% respectively by day 14 (Figure 1).

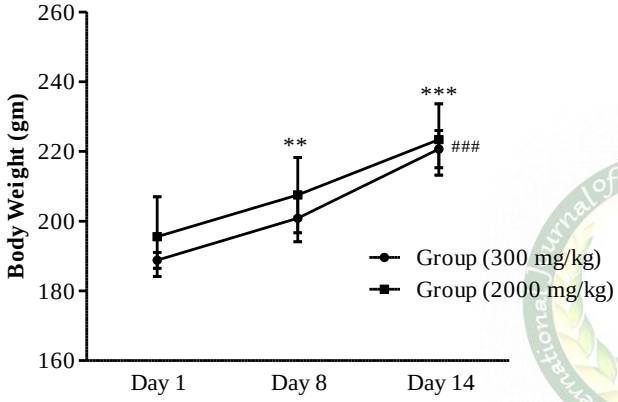

Figure 1: Mean body weight changes (g) in Wistar rats following single oral administration of 300 and 2000mg/kg Amrith Noni Artho Plus at days 8 and 14 of post-treatment.

Clinical signs and mortality

No mortality or evident toxicity was observed at any dose level. Clinical monitoring revealed no abnormal behavioural, neurological, or autonomic signs during acute (0–6 h) or subacute (14-day) phases. All animals remained active with normal grooming, posture, and exploratory behaviour. The absence of tremors, convulsions, lacrimation, or salivation supported the safety profile of the formulation. No mortality during 14-day study period confirmed 100% survival across all groups.

Necropsy and gross pathology

Gross pathological examination post-euthanasia revealed no abnormalities in vital organs (liver, kidneys, heart, lungs, spleen, gastrointestinal tract). Organ weights and macroscopic morphology fell within normal physiological ranges, with no evidence of discoloration, lesions, or hypertrophy. These findings (Figure 2) aligned with clinical observations, further substantiating the non-toxic nature of Amrith Noni Artho Plus at tested doses.

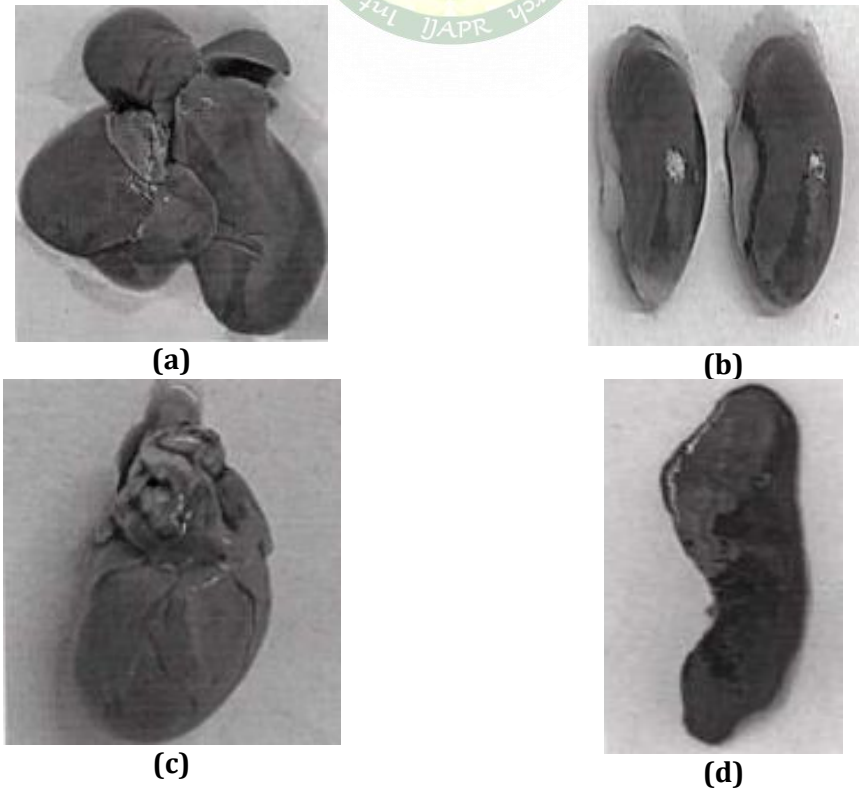

Figure 2: Gross pathology of (a) liver, (b) kidney, (c) heart, and (d) spleen of treated animals (Macroscopic observation)

DISCUSSION

The present study evaluated the acute oral toxicity of Amrith Noni Artho Plus, a polyherbal Ayurvedic formulation containing *M. citrifolia*, *V. negundo*, *B. serrata*, and *C. wightii*, in female Wistar rats following OECD Guideline 423. The results demonstrated no mortality, clinical signs of toxicity, or gross pathological abnormalities at doses up to 2000mg/kg, suggesting a favourable safety profile for this formulation. These findings align with the traditional use of these herbs in Ayurveda and contribute to the growing body of scientific evidence supporting the safety of polyherbal formulations.

The absence of mortality and clinical signs at both tested doses (300 and 2000mg/kg) is significant, as the 2000mg/kg dose represents the limit test threshold per OECD 423 for classifying substances as "Category 5" or "unclassified".[5] This indicates that Amrith Noni Artho Plus is unlikely to pose acute toxicity risks at physiologically relevant doses. The normal body weight gain patterns observed throughout the study further corroborate this conclusion, as weight loss or stagnation often serves as an early indicator of systemic toxicity.[12] The stable food consumption across all groups additionally suggests no adverse effects on appetite or metabolic function, which is consistent with previous safety studies on the individual herbal components. [13-15]

The safety of *M. citrifolia* (Noni) has been well-documented in preclinical studies, with no reported toxicity at doses up to 5000mg/kg in rodents.[16] Similarly, *B. serrata (Shallaki)* and *C. wightii (Guggulu)* have demonstrated safety in acute and subacute toxicity studies at doses comparable to those used in this study.[17,18] However, the novelty of this study lies in its evaluation of the combined formulation, addressing potential herb-herb interactions that could alter toxicity profiles. The absence of synergistic or additive toxicity in Amrith Noni Artho Plus supports the concept of *"Yogavahi"* (synergistic compatibility) described in Ayurvedic texts, where carefully selected herb combinations enhance efficacy without increasing toxicity.[19]

The gross pathological findings showed no abnormalities in vital organs-align with the clinical observations and are further supported by existing literature on the hepatoprotective and nephron-protective effects of the constituent herbs. [20-23] The lack of macroscopic lesions in this study suggests that these protective mechanisms may extend to the polyherbal formulation, though future histo-pathological analyses could provide deeper insights into cellular-level effects.

The study adhered to OECD 423 guidelines, which prioritize animal welfare through the use of minimal animal numbers and humane endpoints.[5] The consistent results across both steps (initial and confirmatory tests) enhance the reliability of the findings, meeting regulatory standards for acute toxicity assessment. The acute toxicity studies are essential for initial safety profiling, however, subchronic and chronic toxicity evaluations are recommended to rule out long-term effects, particularly for herbal medicines intended for prolonged use.

A limitation of this study is the absence of histopathological and biochemical analyses, which could have provided additional data on organ-specific toxicity (e.g., liver enzymes, renal function markers). Future studies should incorporate these parameters, along with mechanistic investigations into the pharmacodynamics of the formulation. Additionally, expanding the dose range beyond 2000mg/kg could establish a more comprehensive safety margin, though the current results already support its classification as a low-toxicity substance.

CONCLUSION

The acute oral toxicity study in Wistar rats confirmed that Amrith Noni Artho Plus is non-toxic at doses up to 2000mg/kg, supporting the established safety of its key components. These findings validate its traditional use and support its potential as a safe therapeutic option for inflammatory and musculoskeletal disorders.

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